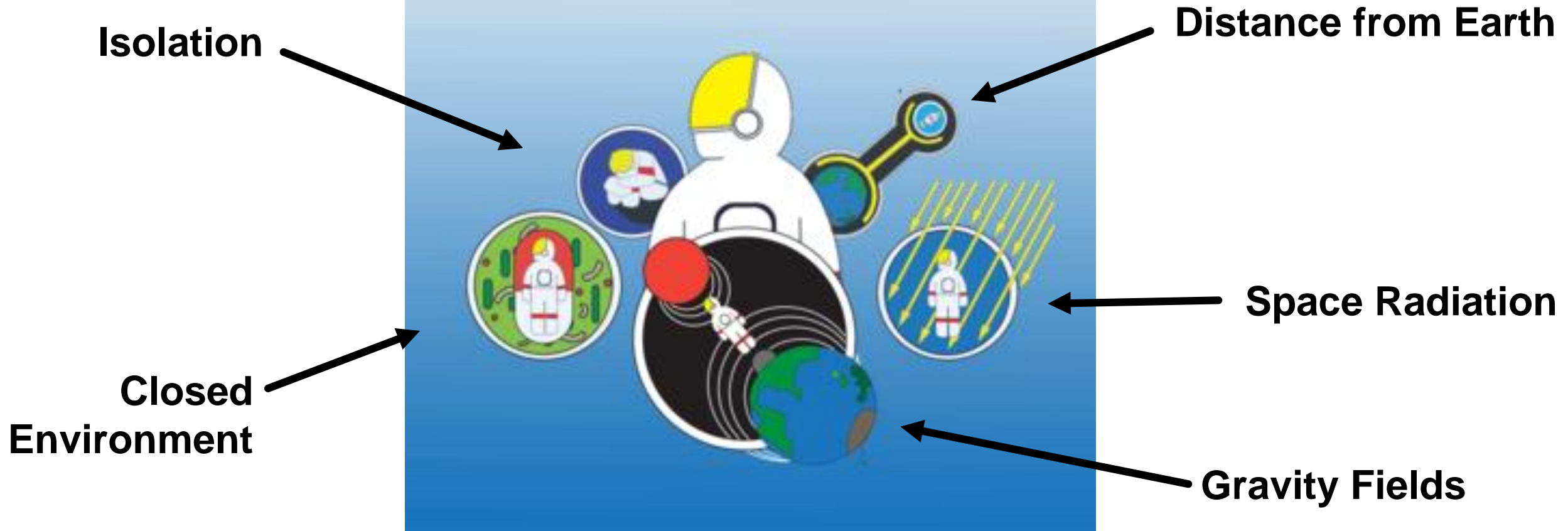


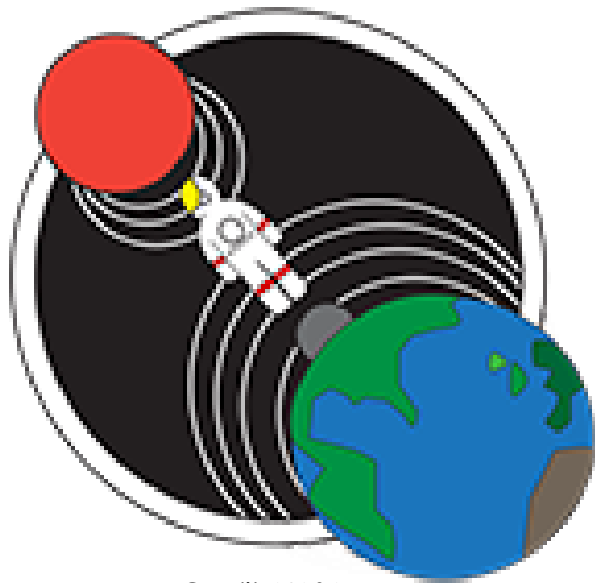
# The Transcriptional Response of Diverse *Saccharomyces cerevisiae* Strains to Simulated Microgravity

LILY S. NEFF<sup>1,2</sup>, SAMANTHA T. FLEURY<sup>3,4</sup>, JONATHAN M. GALAZKA<sup>5</sup>

# Stresses of Spaceflight

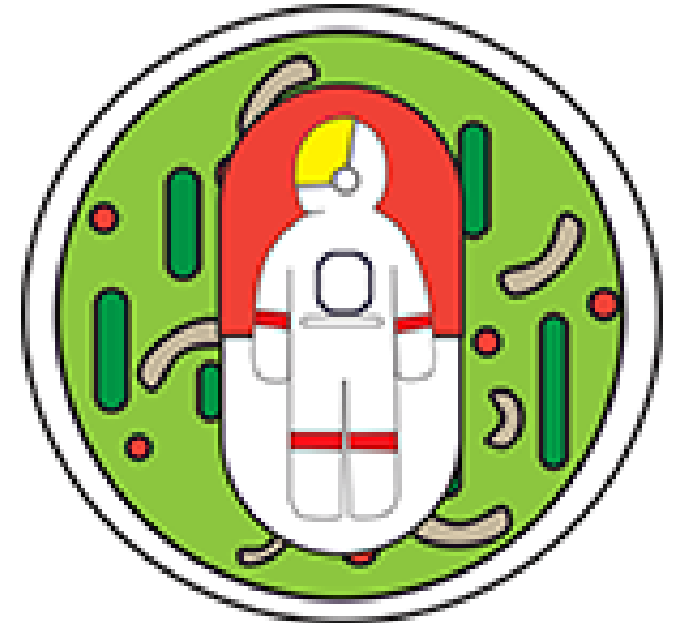


# Microgravity



Credit: NASA

- ▶ Space exploration missions place stresses on the space crew and their supporting microbial commensals
- ▶ Reveal a conserved response to the stress of microgravity, measure physiological response



Credit: NASA

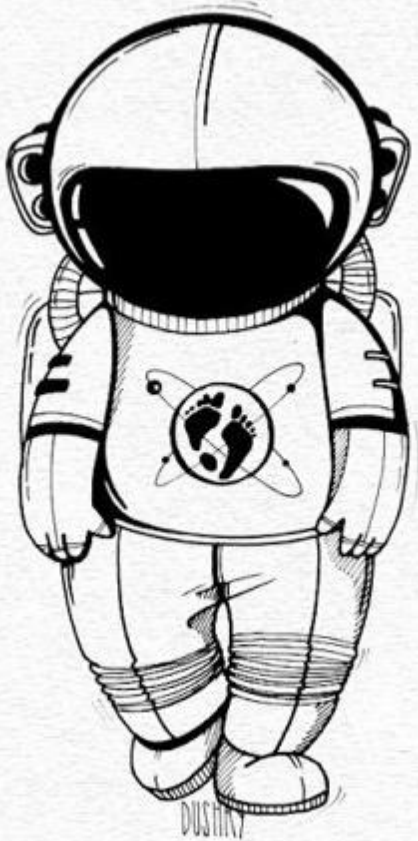
# Why yeast? Why *S. cerevisiae*?

## Yeast

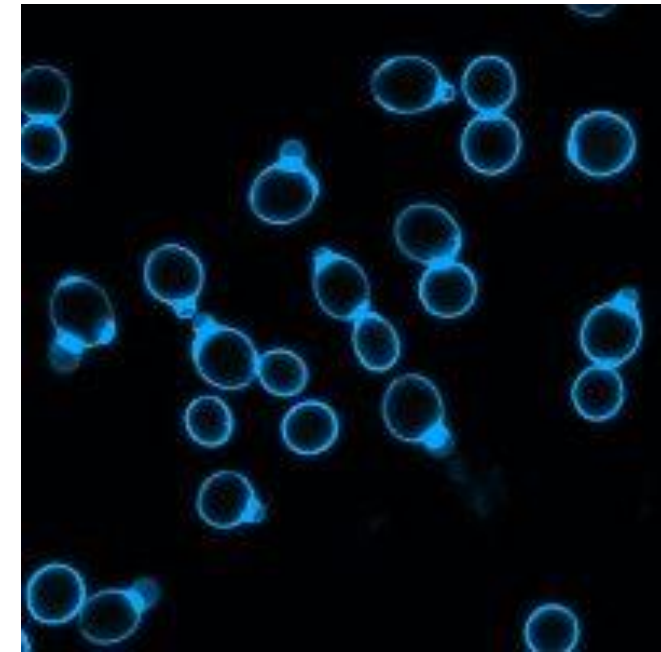
- ▶ Powerful microbial model
- ▶ Easy to grow and allows for transcriptomes to be recorded cheaply
- ▶ Part of human microbiota

## *S. cerevisiae*

- ▶ Human colonizer
- ▶ Opportunistic pathogen
- ▶ Diverse set of strains are readily available



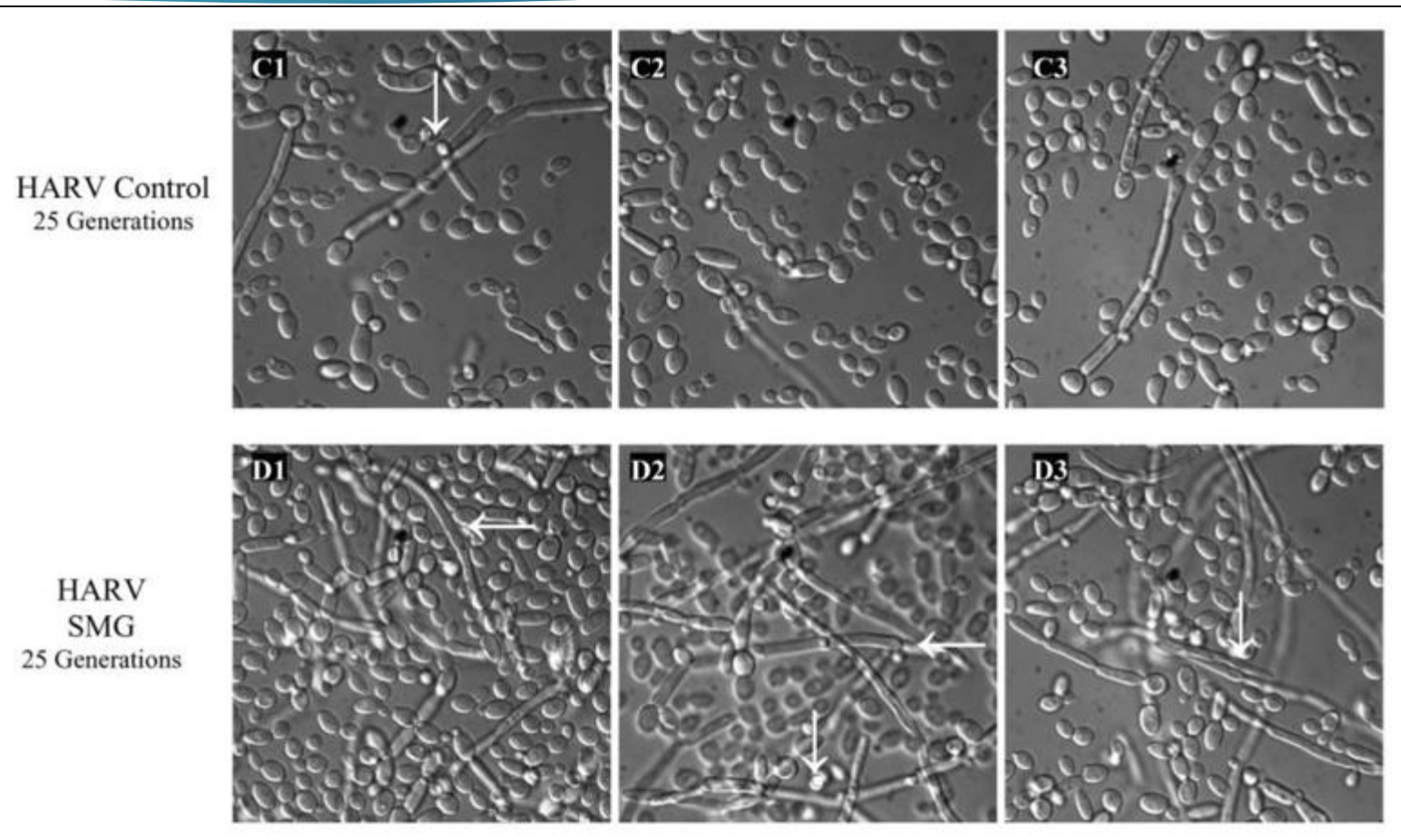
Credit: Dushky



Credit: Biotium

# Studying Simulated Microgravity

- ▶ Environmental stress of SMG causes an increased growth of *Candida albicans* in filamentous forms<sup>1</sup>
  - ▶ Alteration in two genes associated with this hyphal transition
  - ▶ Evidence of enhanced pathogenicity, fungal pathogen becomes more virulent
- ▶ ~300 million year divergence from *S. cerevisiae*<sup>2</sup>



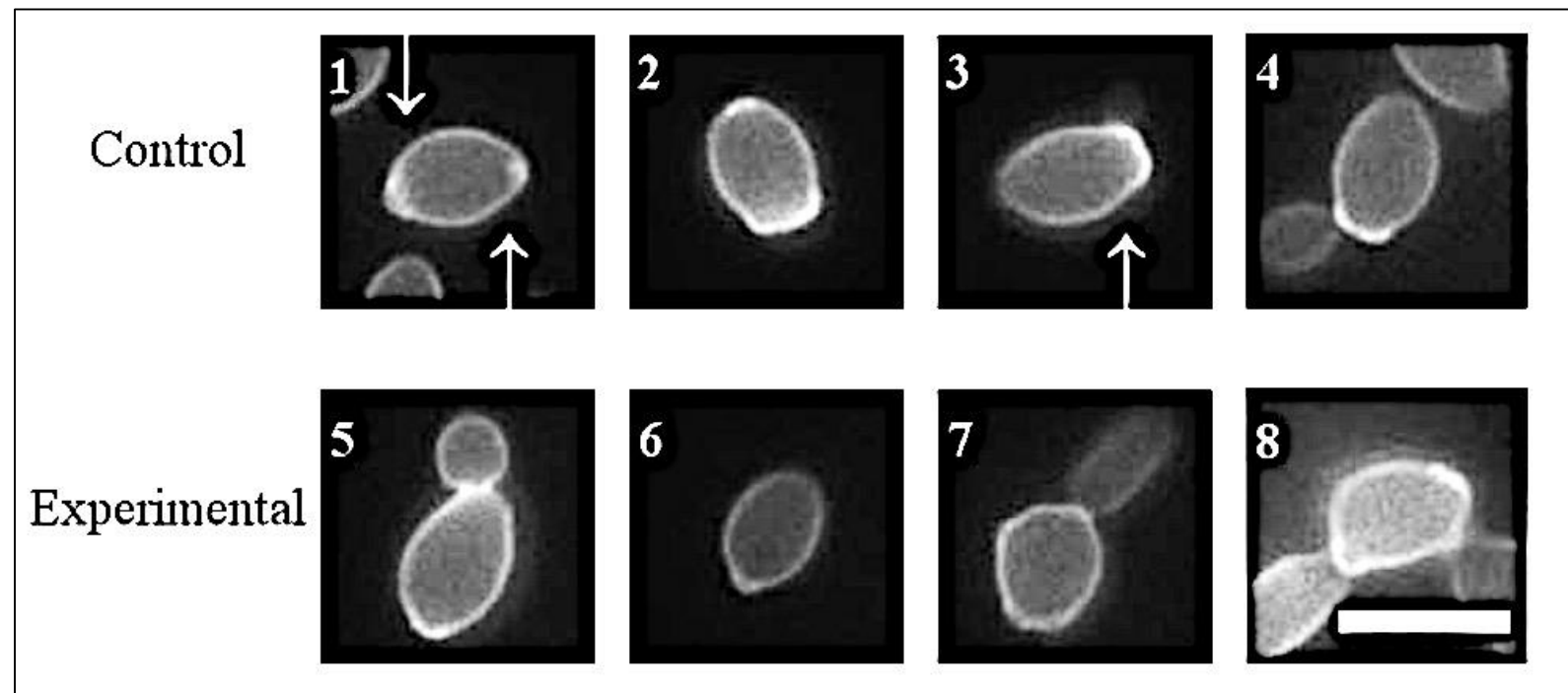
<sup>1</sup>(Altenburg et al. 2008 Geno. Prot. Bioinfo. Increased Filamentous Growth of *Candida albicans* in Simulated Microgravity)

<sup>2</sup>(Hedges SB et al., Tree of life reveals clock-like speciation and diversification. Mol Biol Evol. 2015 Apr32(4):835-45.)

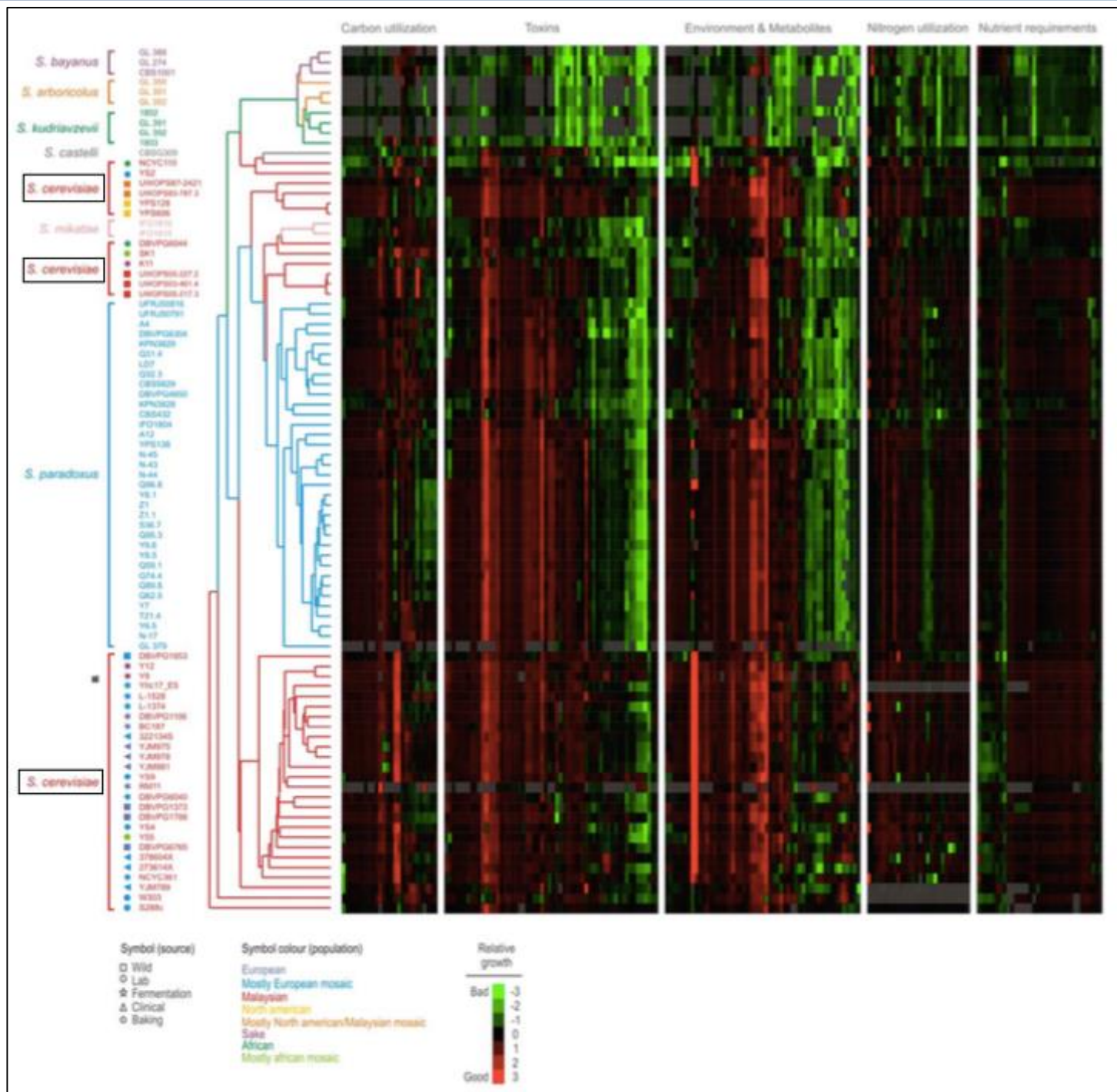


# Studying Simulated Microgravity cont.

- ▶ Studies conducted show that cells perceive and respond to variations in mechanical forces, i.e. gravity<sup>1</sup>
- ▶ *S. cerevisiae*, under SMG, demonstrates random budding than typical bipolar budding pattern<sup>1</sup>

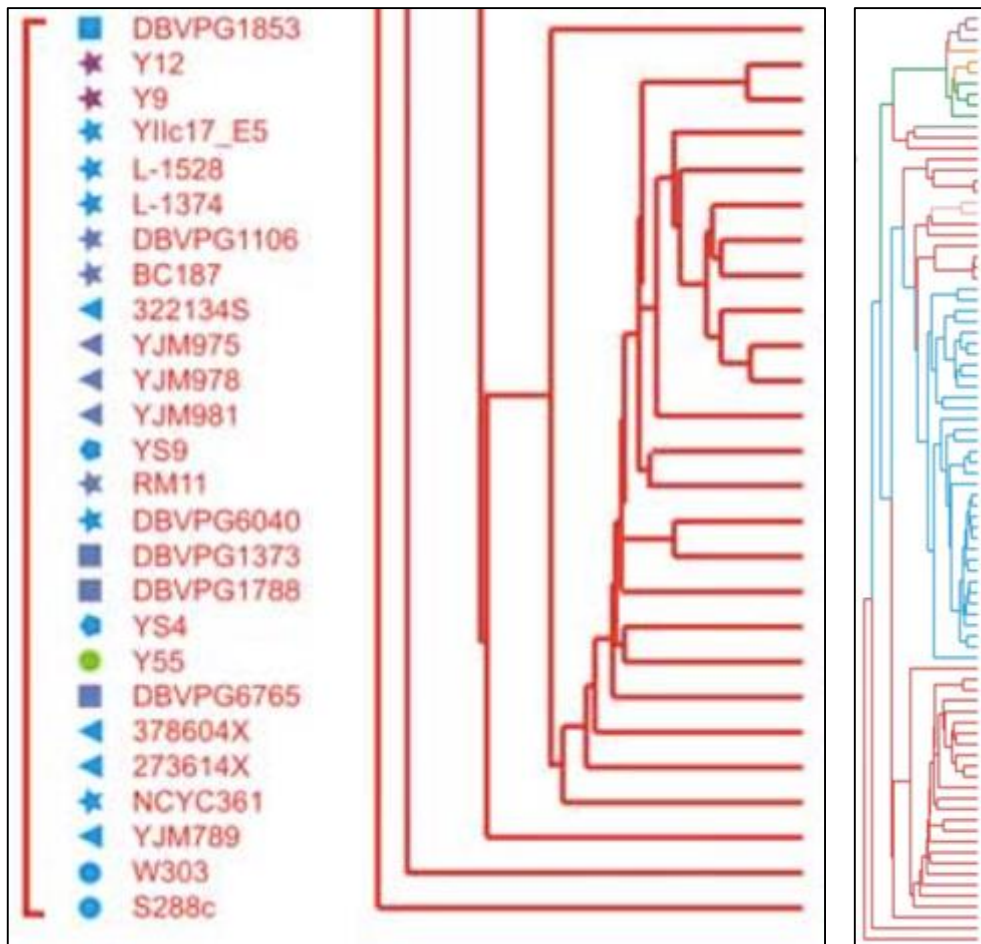
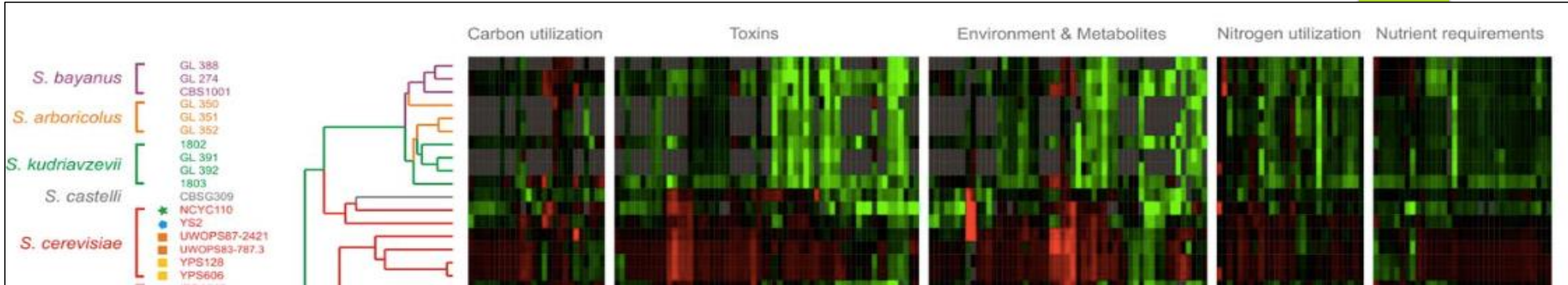


<sup>1</sup>(Sheehan et al. 2007 BMC Genomics. Yeast genomic expression patterns in response to low-shear modeled microgravity)



Hierarchical clustering based on 600 growth rate variables

- ▶ clustering represents patterns
- ▶ shows diversity in phenotype and physiology

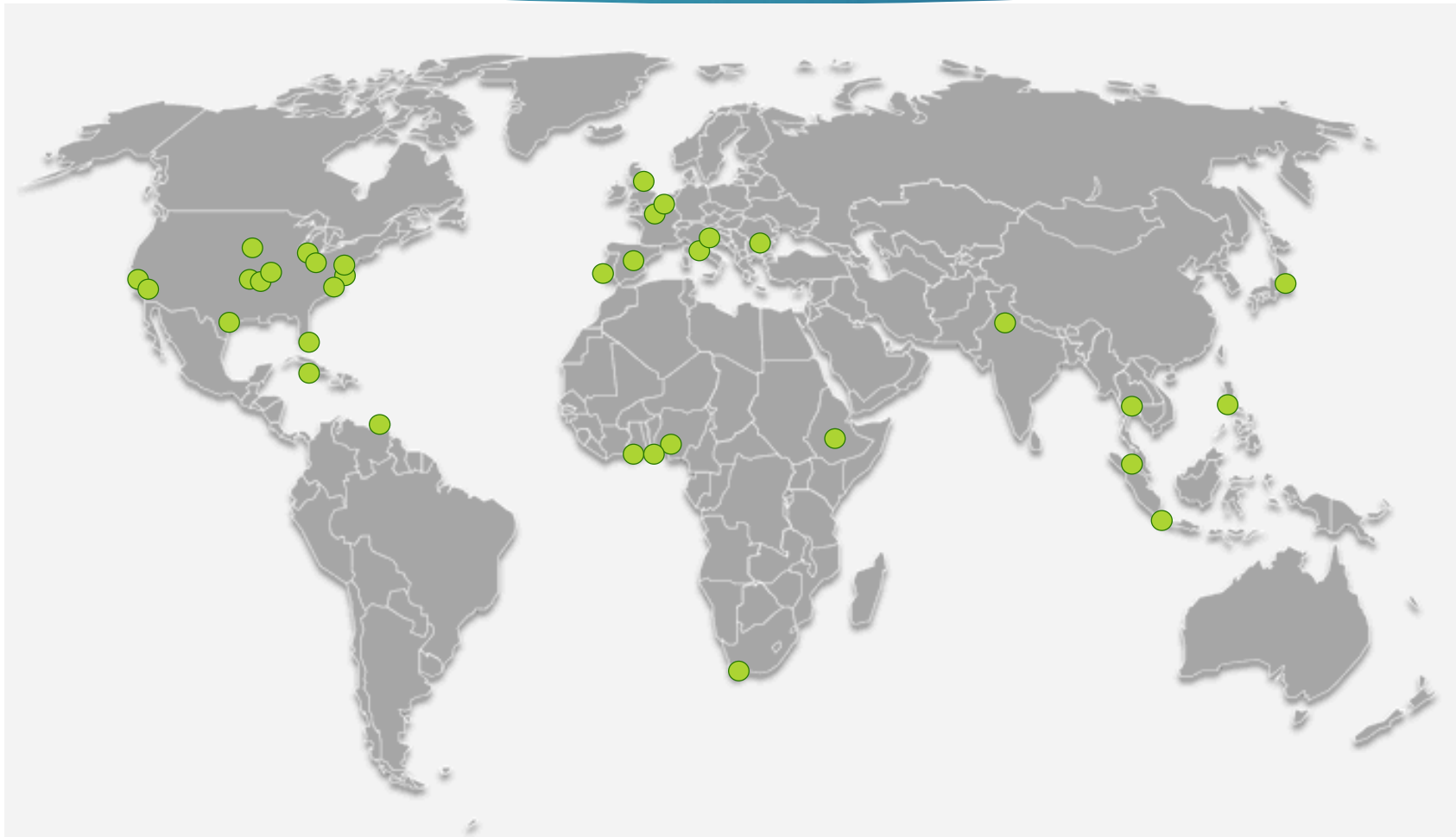


## S288c

- *Saccharomyces sensu stricto* isolates
- Hierarchical clustered based on proliferation rates for 600 traits
- Studies on lab strain, S288c
- Species are indicated by line color, population by symbol color



# Yeast are Diverse!



# Project Work Flow

- ▶ Central Objective: Reveal a conserved response across all strains or unique to lab strain, S288c

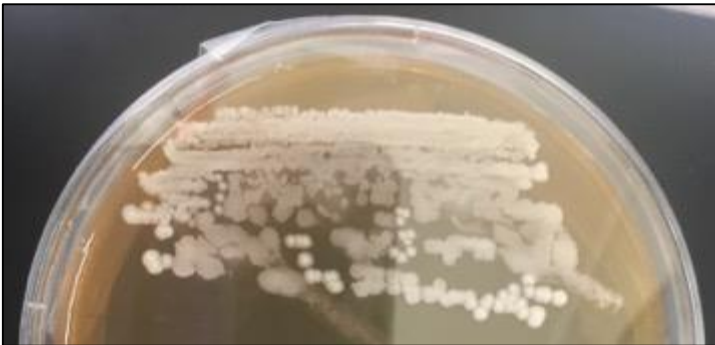
- ▶ How to accomplish this:



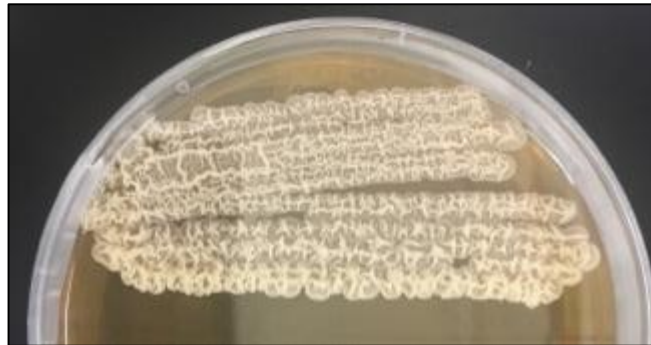
# Screening Procedure: YPD Plate

- ▶ Create YPD plates (1% yeast extract, 2% peptone, 2% glucose) and YPD liquid culture
- ▶ Inoculate YPD plates with strains from cryogenic stock
- ▶ Observe for unusual growth (different morphologies):

YJM981 (flattened)



YJM1401 (wrinkled)

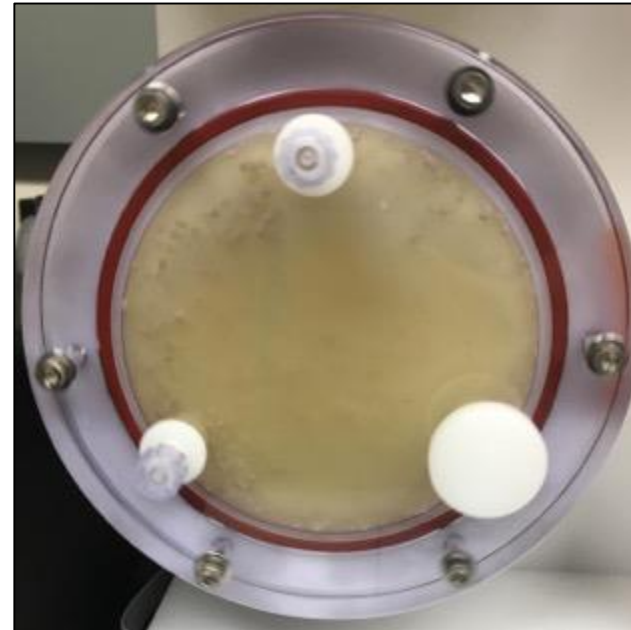


YJM996 (normal)



# Screening Procedure: Liquid Culture

- ▶ Inoculate 5mL YPD broth from overnight “normal” cultures on YPD plates, incubate overnight
- ▶ Dilution 10 $\mu$ L:100 $\mu$ L to test OD<sub>600</sub> using NANODROP 2000 Spectrometer
- ▶ 24 hour incubation for microscopy check
- ▶ 48 hour incubation for HARV Vessels



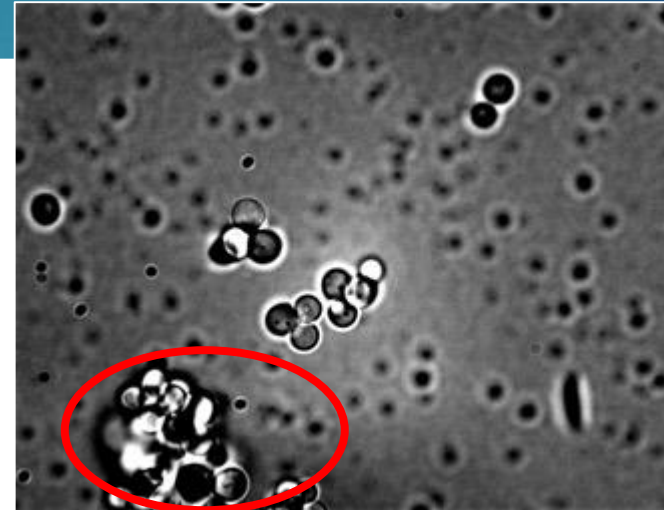
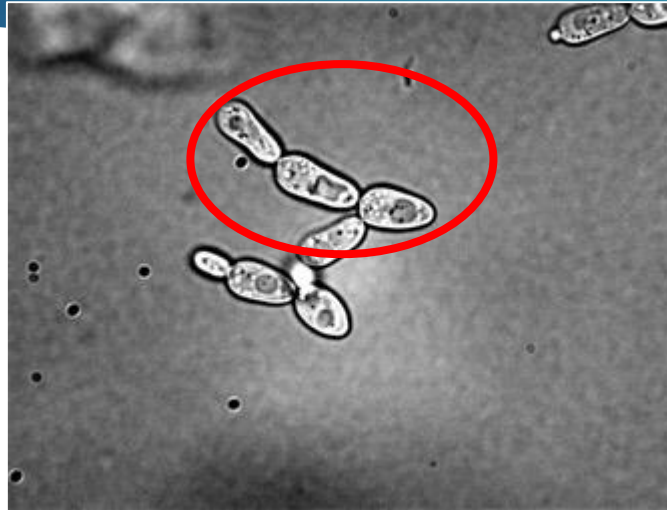


# Screening Procedure: Microscopy

Top Row (left to right):

YJM1439 (West African, Clinical)

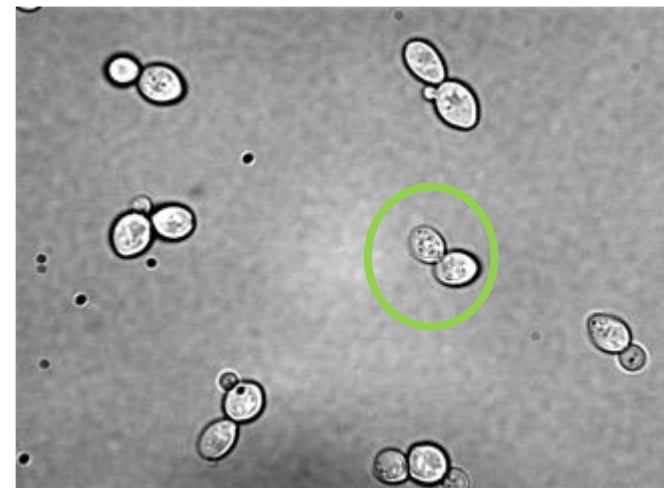
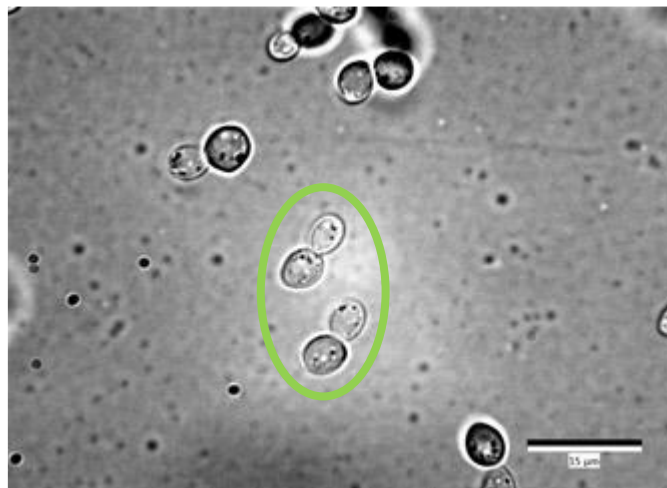
YJM1388 (Sake, Non-clinical)



Bottom Row (left to right):

YJM1248 (West African, Non-clinical)

YJM627 (West African, Non-clinical)



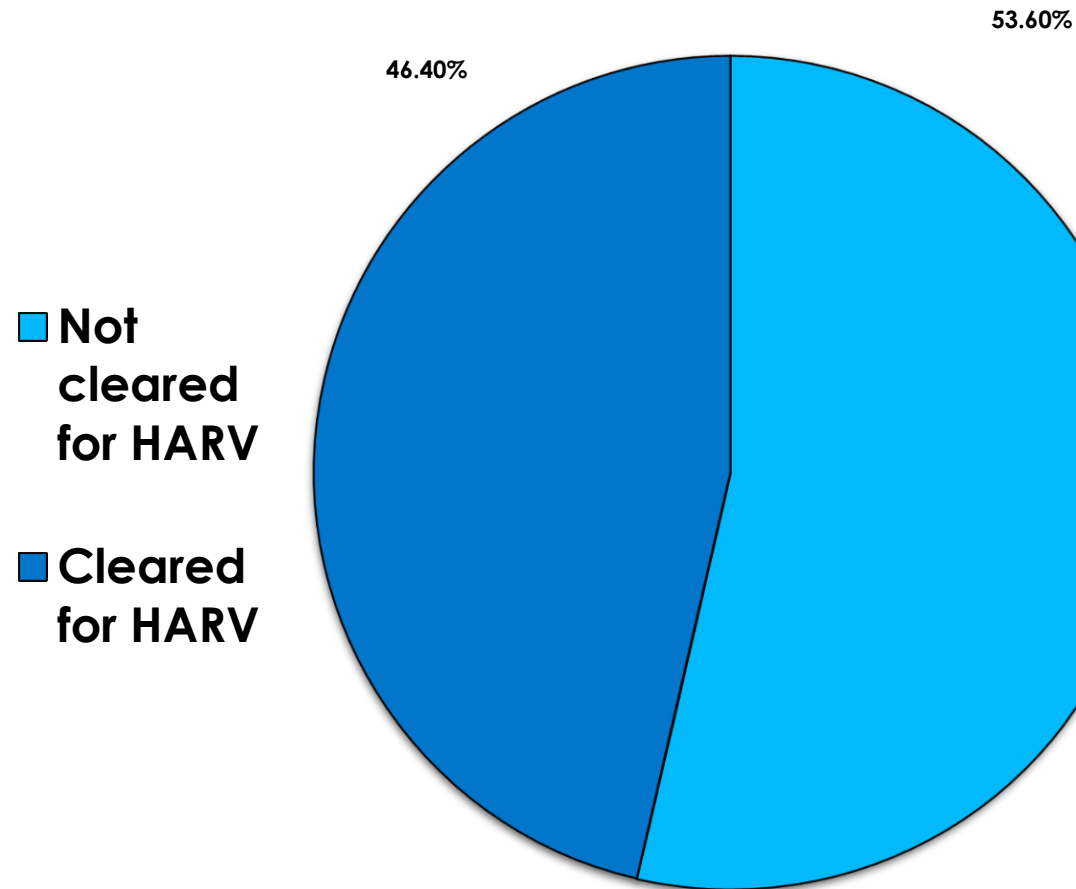
# Results to Date

## Color Code Key:

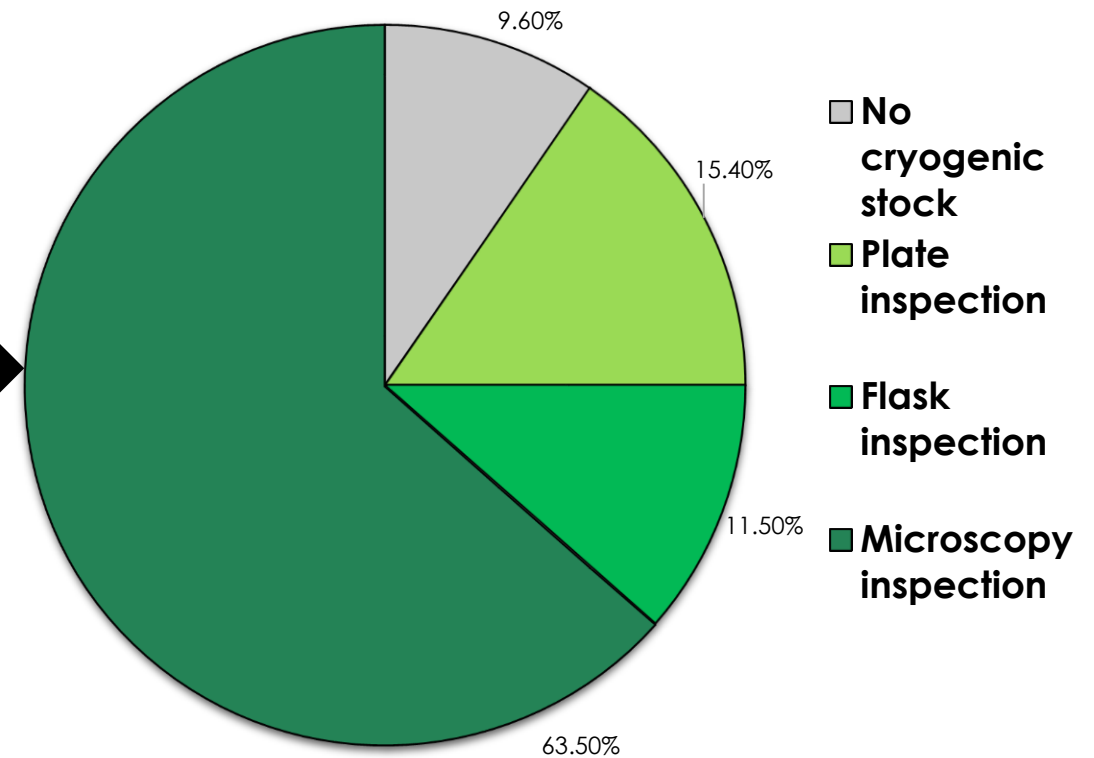
- ▶ **Gray:** cryogenic stock DNE
- ▶ **Red:** Did not have normal phenotype, cannot use
- ▶ **Light Green:** Normal Phenotype so far; TBD
- ▶ **Dark Green:** All normal, including Microscopy Phenotype; can use in HARV vessel

Strain	Clade	Plate	Flask	Microscope	Cleared for HARV
YJM1078	European, Clinical				
YJM1450	European, Clinical				
YJM1526	European, Clinical				
YJM244	European, Clinical				
YJM248	European, Clinical				
YJM453	European, Clinical				
YJM969	European, Clinical				
YJM972	European, Clinical				
YJM978	European, Clinical				
YJM984	European, Clinical				

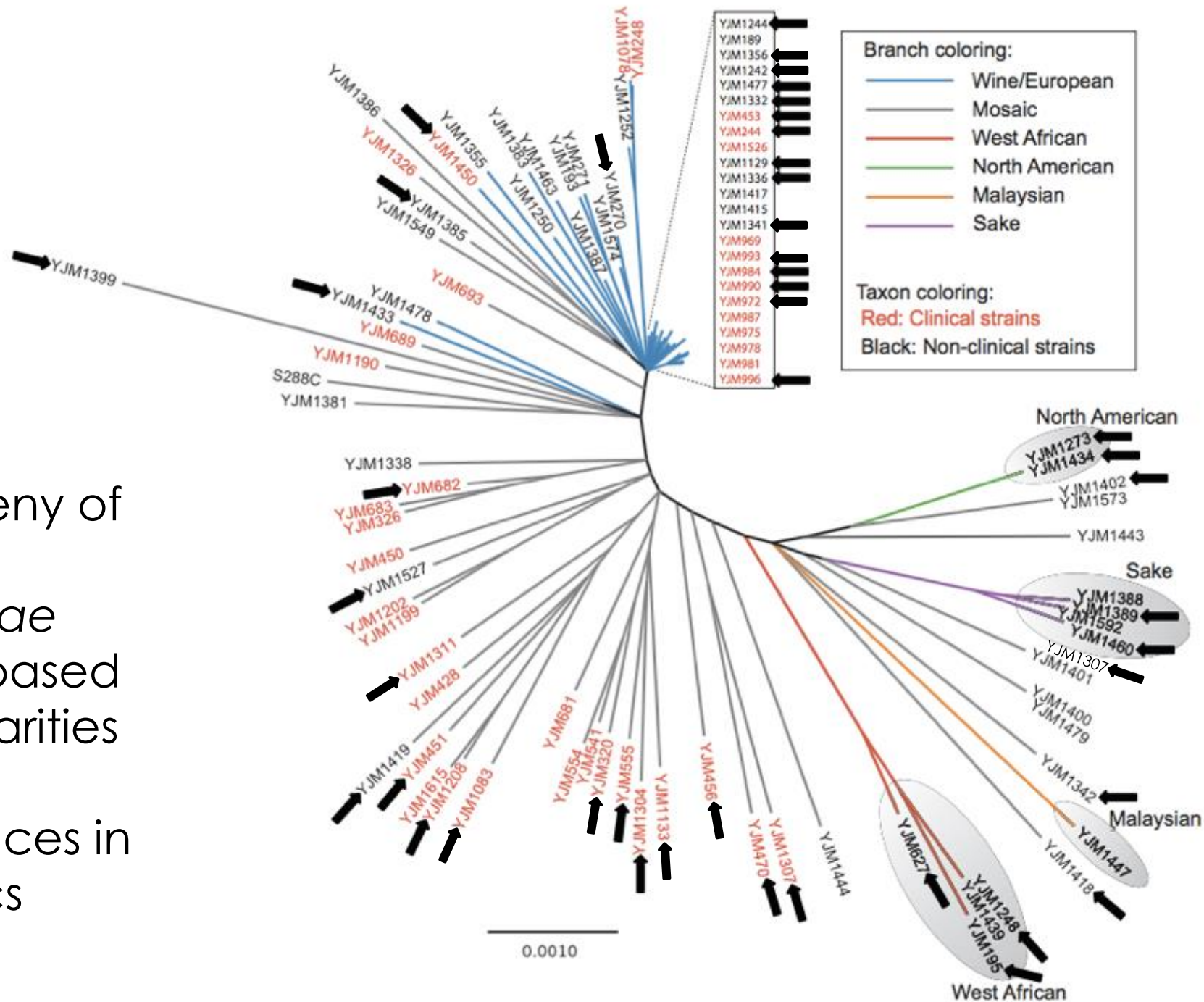
# HARV Vessel Screening



## Not cleared for HARV: Screening Stages



► Phylogeny of 94 *S. cerevisiae* strains based on similarities and differences in genetics

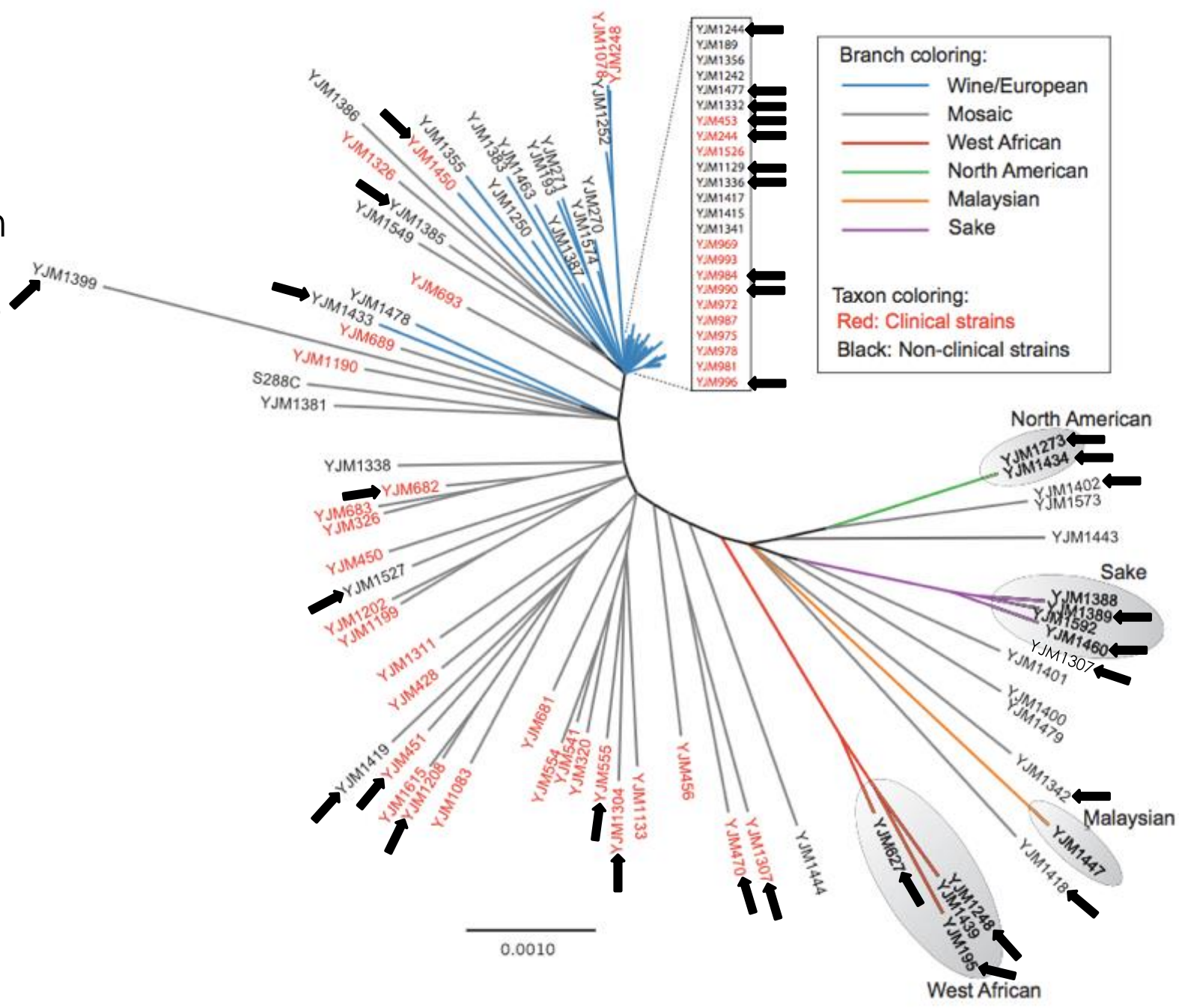


Cleared  
for  
HARV  
Use



► Selected 34 *S. cerevisiae* strains:

- isolated from clinical and environmental settings
- multiple locations around the world to encompass evolutionary divergence

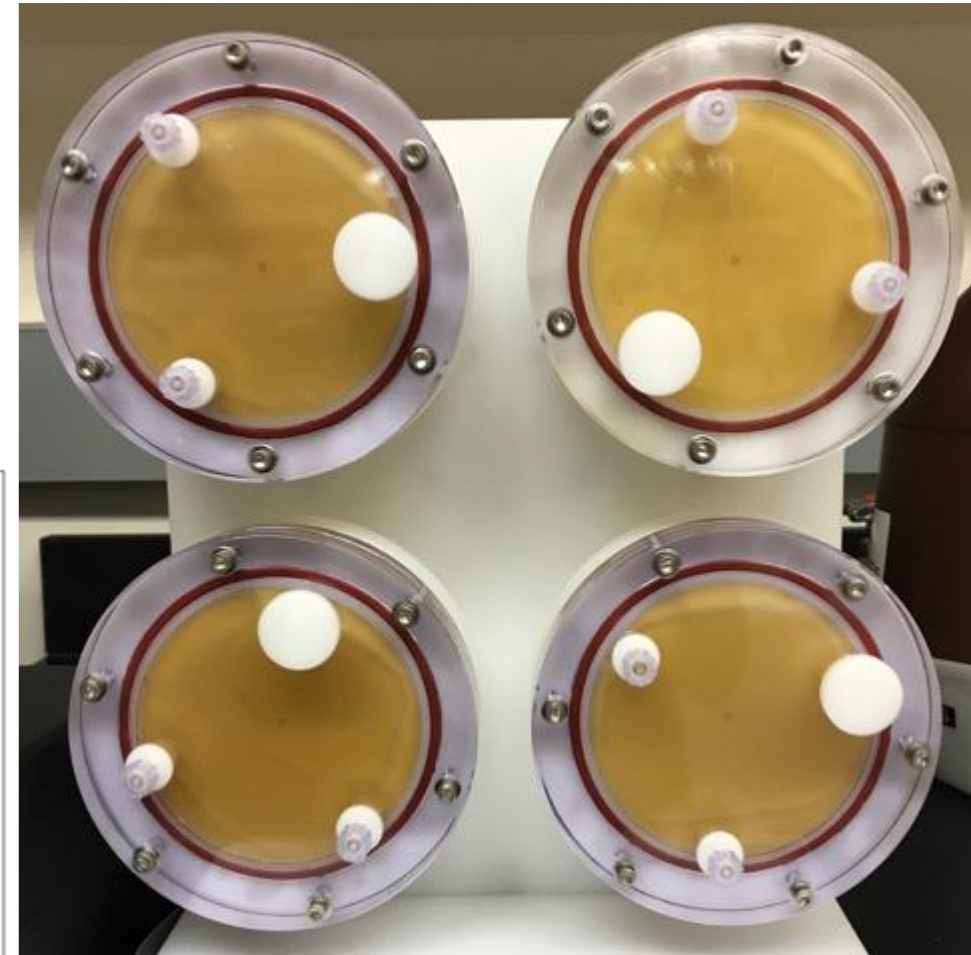
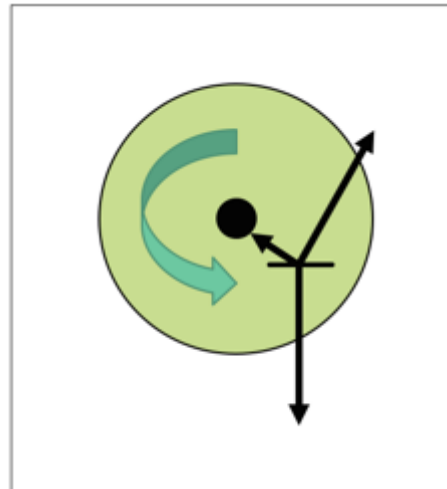


Strains  
to  
Study

# High Aspect Ratio Vessel (HARV)

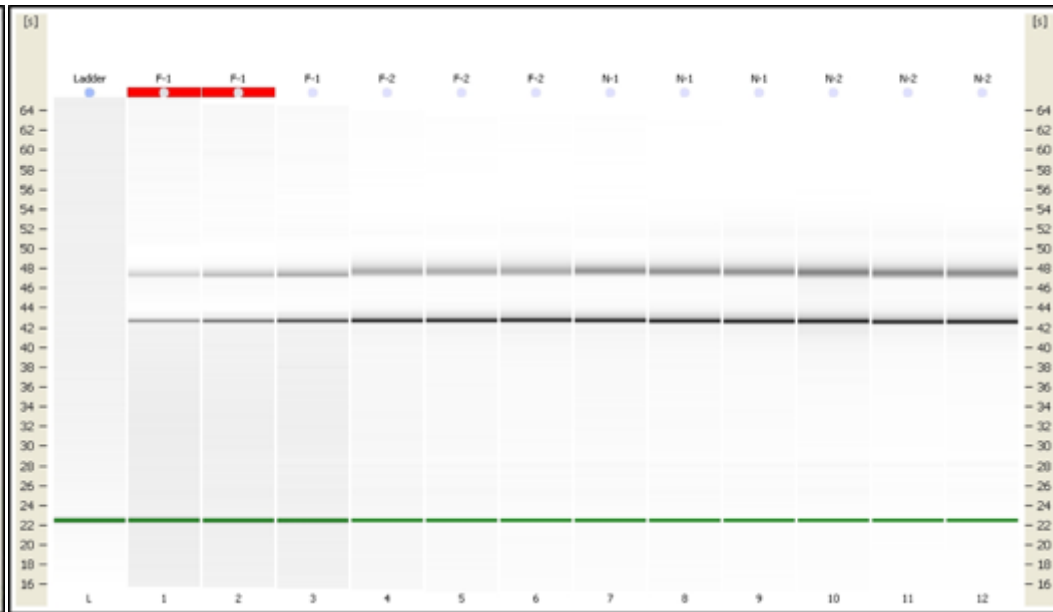
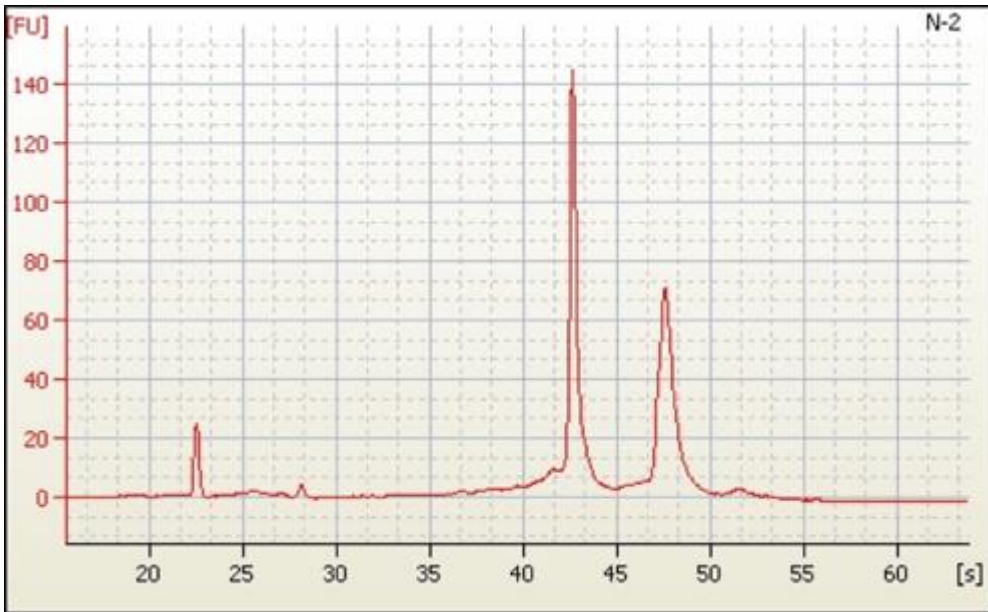
- ▶ Simulates microgravity conditions by rotating on vertical plane
- ▶ “functional weightlessness”\*
  - ▶ randomizes the gravitational effect
  - ▶ minimizes turbulence (fluid undergoes irregular fluctuations) over surface of cell
- ▶ Remain suspended in liquid culture

\*(Altenburg et al. 2008 *Geno. Prot. Bioinfo.* Increased Filamentous Growth of *Candida albicans* in Simulated Microgravity)

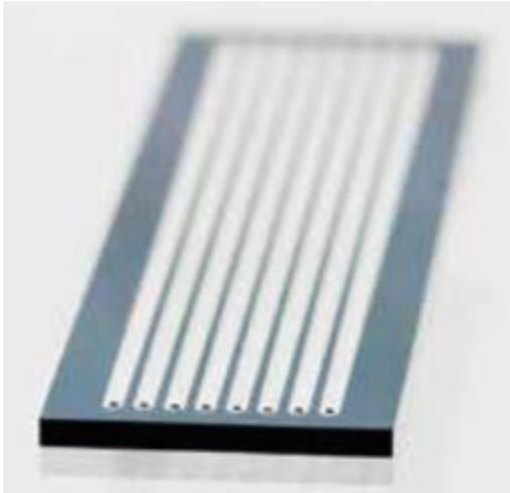


# NANO Chip Bioanalyzer

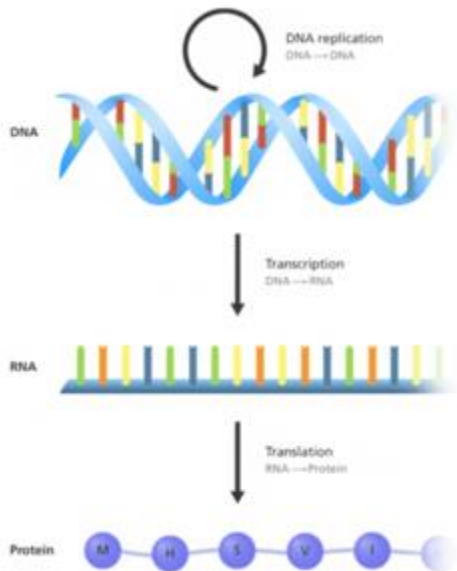
- ▶ Purity (degree of contamination) and quality (intactness/integrity) of RNA are essential for examining gene expression
- ▶ Degraded samples lead to misrepresentative data and inconsistency in reproducibility



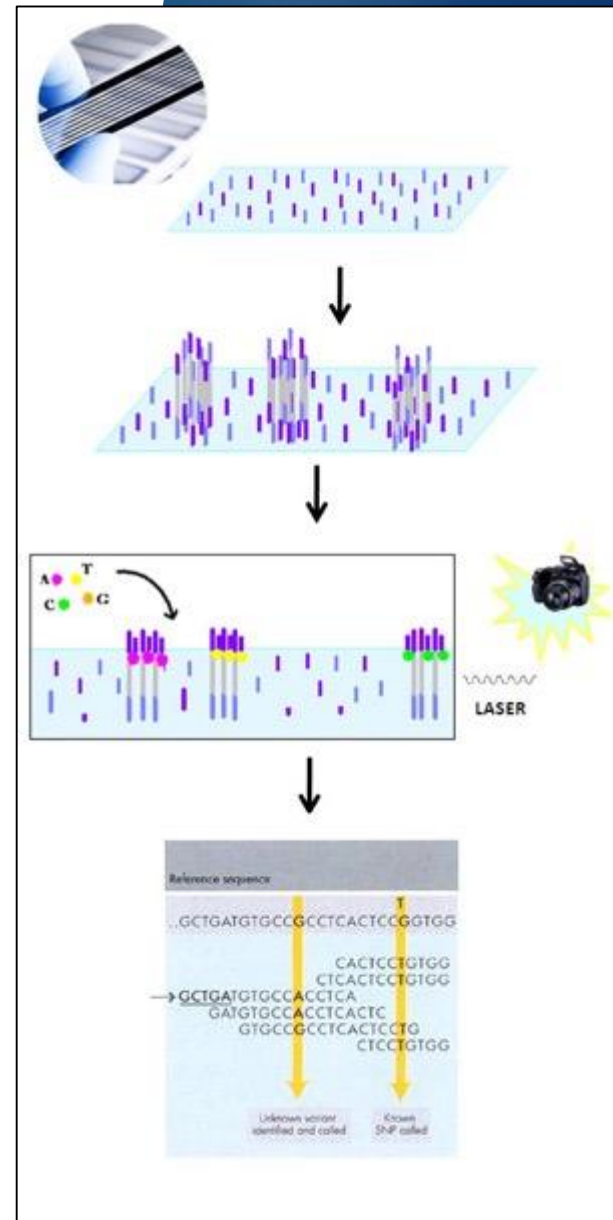




- ▶ Allow for generation of transcriptome information cheaply
- ▶ Allows for the investigation of known transcripts and new ones (important for the comparisons)



- ▶ Analyze physiology and phenotype
- ▶ Allows for the identification of conservation with gene expression profiles



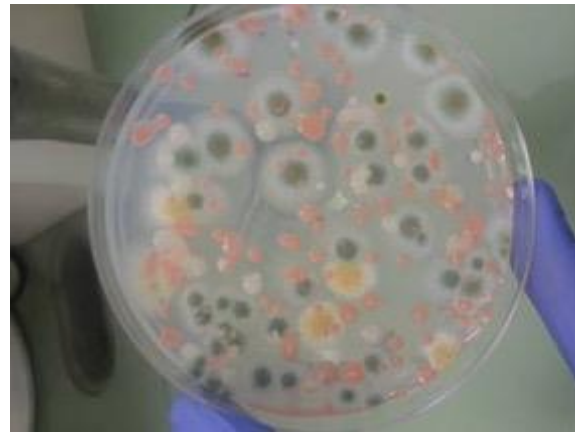


# Future Plans of Progression

- ▶ Complete simulated microgravity runs for the 32 strains (along with control experiments)
- ▶ Complete RNA Extraction and Illumina sequencing of samples using the KAPA mRNA HyperPrep Kit
- ▶ Send samples to be sequenced at UCSF and analyze data

# Significance

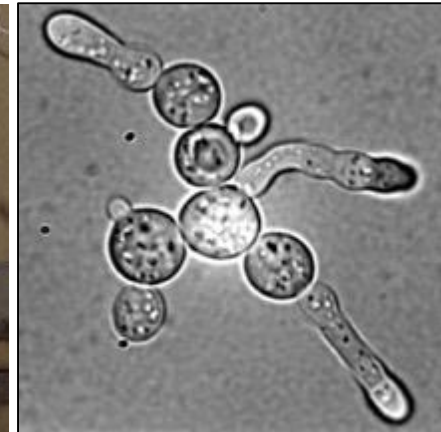
- ▶ Systemic understanding of how microbes respond to simulated space flight environment



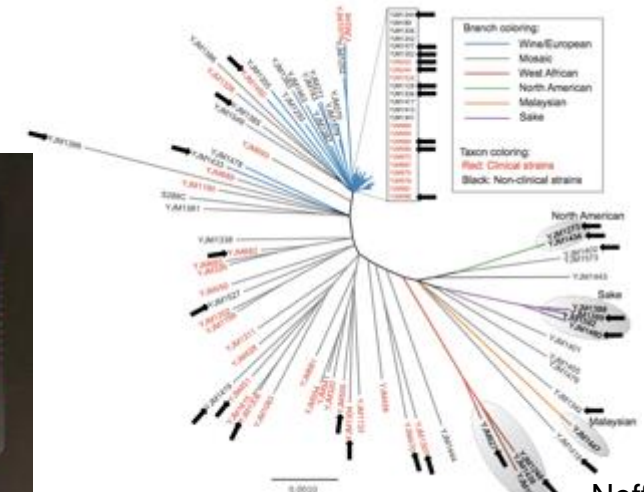
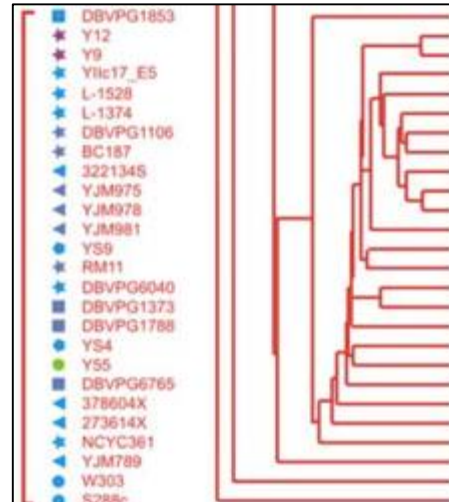
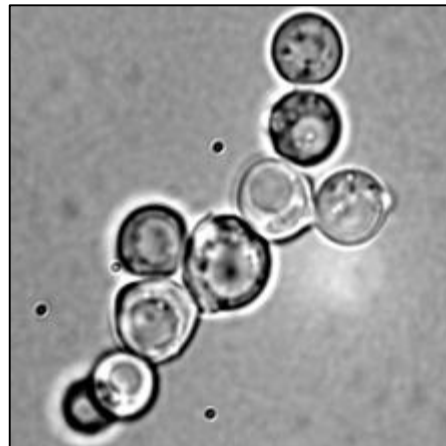
Credit: NASA



Credit: NASA



- ▶ Serve as a platform for future flight experiments



# Acknowledgements

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- ▶ Dr. Asif Rahman (Wyle Labs)
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